(attached). Differences between treatments for AUC and AUCINF were 9% (B>A) and there was a 10.4% difference in CMAX (B>A). There were statistically significant ($p < 0.05$, B>A) treatment effects for AUC, AUCINF, and CMAX. The reported 90% C.I. for AUC, AUCINF, and CMAX are all within the allowed equivalence interval.

16. Mean blood levels of chlorthalidone resulting from both treatments as reported by the sponsor are shown in Table 6 (attached). At all times except 96 hr, blood levels from Trt. A exceeded levels from Trt. B. During the 0.5-2 hr interval, differences between treatments were > 27% (A>B). There were statistically significant ($p < 0.05$, A>B) treatment effects at 0.5, 1, 2, 3, 4, 6, 10, 12, 18, 24, and 36 hr, and a significant ($p < 0.05$) period effect at 0.5 hr. There were statistically significant ($p < 0.1$) sequence effects at 1, 2, 3, 4, 8, 14, 16, 18, 24, 48, and 72 hr.

There were nonzero predose chlorthalidone blood levels for S5 in both periods. The sponsor set these values to 0.0 since they were < 5% of the CMAX.

17. Mean pharmacokinetic parameters of chlorthalidone resulting from both treatments as reported by the sponsor are shown in Table 7 (attached). Differences between treatments for AUC, AUCINF, and CMAX were < 6%. There were statistically significant ($p < 0.05$, A>B) treatment effects for AUC, CMAX, and KEL, and significant ($p < 0.1$) sequence effects for AUC ($p = 0.0645$) and CMAX ($p = 0.0838$).

From Comment #10 above, using the reviewer's revised chlorthalidone data, the reviewer also observed significant sequence effects for AUC ($p = .0708$), logAUC ($p = .0658$), CMAX ($p = .0849$), and logCMAX ($p = .0775$). There were nonzero (> LLOQ) predose chlorthalidone blood levels for subjects 5 and 6 in Period 2 (79.49 and 65.13 ng/ml, respectively), and for subject 5 in Period 1 (90.66 ng/ml). It should be noted that the lowest acceptable standard for curve ANQ05 was 500 ng/ml by the reviewer's criteria; however, the sponsor's practice in this study was to accept chlorthalidone blood levels above the lowest standard (50 ng/ml) that was validated for the assay (see Comment #10 above).

18. The Period 1 chlorthalidone blood concentration-time data for subjects 5 and 6 was analyzed by curve-stripping and nonlinear regression to accurately determine the terminal phase $t_{\frac{1}{2}}$. The ESTRIP function of the PKCALC program (Shumaker, Drug Metab. Rev., 17: 331, 1986) was used to strip each curve into a sum of exponentials and to generate initial estimates for pharmacokinetic parameters; in both cases, two terms were optimal. PCNONLIN V. 3.0 was then used to fit (simplex method) each data set to a biexponential equation (one-compartment model with first-order input and time lag) using the initial estimates for absorption rate constant (KA), elimination rate constant

(KE), and time lag (TL), and a calculated estimate for volume (V). The results are shown in Table 8 (attached); to maintain consistency in units, blood levels are expressed in mg/L, dose in mg, and AUC in mg-hr/L.

From Table 8, it is apparent that the washout interval for S6 was less than the usual minimum of seven half-lives; direct drug carryover may have partly contributed to the predose levels in Period 2. However, the washout for S5 appears adequate, and the predose levels in Period 1 for S5 further complicates any explanation. The apparently low volumes of distribution (Vd) for these subjects in Table 8 probably reflect the very high binding of chlorthalidone to erythrocytes. Reported values are 3.9 L/kg for Vd and 72.5 for RBC/plasma partition ratio (Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th ed., p. 1669) and 4.1 L/kg for Vd (Ritschel, Handbook of Basic Pharmacokinetics, 3rd ed., p. 500). The blood sample processing procedure used in this assay may not effect a complete release of chlorthalidone from its RBC binding sites.

19. The labeling for Tenormin® (PDR, 1992, p. 1107) indicates that TMAX occurs at 2-4 hr after oral dose, and that, following an intravenous dose, plasma level decline is rapid over the first 7 hours; thereafter, plasma levels decline with a $t_{1/2}$ similar to orally administered drug. The reviewer recommends using data points only ≥ 8 hr to estimate KEL and to calculate AUCINF.

Using the REG procedure of SAS®, the reviewer calculated: 1) the terminal elimination phase rate constant $lambda_z$ using all data points at 8 hr and after; 2) $AUC_{0-\infty}$ (= AUC0-t_{LAST} + C_{LAST} / $lambda_z$), using the revised AUC values from Comment #9 above; 3) RATIO (= AUC0-t_{LAST} / $AUC_{0-\infty}$); 4) half-life ($t_{1/2}$ = 0.693 / $lambda_z$); 5) NUMHALF (= T_{LAST} / $t_{1/2}$).

The results are shown in Table 9 (attached). There was a statistically significant period ($p < 0.05$) effect for logAUCINF, and a significant treatment ($p < 0.05$) effect for AUCINF. The 90% C.I. for $AUC_{0-\infty}$ and log$AUC_{0-\infty}$ are both within the allowed equivalence intervals. RATIO was < 0.8 in only one case (S16, Trt. A). NUMHALF was < 3 half-lives in 6 cases.

20. Literature reports (Beermann, Clin. Pharmacokinet., 5: 221, 1980) indicate that chlorthalidone is 50-80 times more highly concentrated in RBC's than in plasma due to high affinity for erythrocyte carbonic anhydrase. Consequently, the plasma kinetics of chlorthalidone after a single oral dose are described by a two-compartment model (three exponential terms) with terminal phase half-lives of 40-65 hr. However, the whole blood kinetics (uptake, nonlinear binding, and elimination in RBC's) of chlorthalidone are described by a one-compartment model (two terms) with terminal phase half-lives of 53-60 hr.

Therefore, the PKCALC program was used to fit each data set to a one-compartment model (two exponential terms) in order to estimate lambda$_z$. Revised values (subjects 1, 2, 5, 6, 7) for AUC and C_{LAST} were used (as described in Comment #10 above) to calculate $AUC_{0-\infty}$. The reviewer recognizes that results from curve-stripping procedures are only intermediate and require nonlinear regression for final values. However, the reviewer still considers the results generated from curve-stripping to be superior to "visual" identification of the terminal phase.

The results are shown in Table 9 (attached). There were no statistically significant period ($p < 0.05$), treatment ($p < 0.05$), or sequence ($p < 0.1$) effects. The 90% C.I. for $AUC_{0-\infty}$ and $logAUC_{0-\infty}$ were both within the allowed equivalence intervals. RATIO was < 0.8 in 21 cases, and NUMHALF was < 3 half-lives in 45 cases. Therefore, $AUC_{0-\infty}$ should be considered the primary bioequivalence parameter for chlorthalidone.

X. Waiver Request for the 50/25 mg Test Product

1. The sponsor has included a formal request for waiver of bioequivalence study requirements for its test product atenolol/chlorthalidone 50/25 mg tablet.

2. Comparative formulations of the biostudy test product (100/25 mg tablet) and the 50/25 mg tablet are shown in Attachment 1. The products are exactly proportional with regard to the active ingredient atenolol and all the inactive ingredients, and contain the same amount of chlorthalidone.

3. Dissolution data for the 50/25 mg test product is shown in Table 1 (attached). The dissolution testing is acceptable.

XI. Deficiencies

1. The sponsor must provide an executed batch record for the biostudy lot of test product (#90-026T) which indicates both the theoretical and finished batch sizes.

2. The sponsor must provide documentation showing the potencies of both test and reference biostudy products.

3. The sponsor should include raw data for the results of autosampler stability for both analytes.

4. The data demonstrating frozen stability of chlorthalidone from studies currently in progress should be submitted.

5. The sponsor should provide details of the analytical method used in dissolution testing.

6. The sponsor should explain its rationale and provide all data and calculations concerning the choice of 1/CONC as weighting factor for standard curves for both analytes.

7. The sponsor should explain why the 50 ng/ml and 1500 ng/ml standards from chlorthalidone standard curve ANQ03 were not rejected since their back-calculated values deviate from nominal values by -36% and 20.7%, respectively. Similarly, the 50 ng/ml and 100 ng/ml standards from curve ANQ05 deviate by -24.8% and 28.9%, respectively. The sponsor should explain why these standards were also not rejected.

8. The sponsor should explain the presence of nonzero predose chlorthalidone levels in subject 5 for both periods of the study.

9. The sponsor should explain the observed statistically significant ($p < 0.1$) sequence effects for chlorthalidone AUC and CMAX.

10. The sponsor's reported chlorthalidone data indicates mean values of approximately 0.80 for both treatments for the ratio $AUC_{0\text{-}TLAST}$ / $AUC_{0\text{-}\infty}$, and that blood sampling occurred over approximately 2.5 half-lives (120 hr / 50 hr) for both treatments. Therefore, the sponsor should use state-of-the-art nonlinear regression methods for accurate determination of the KEL values used to calculate $AUC_{0\text{-}\infty}$ for each data set. The revised values of $AUC_{0\text{-}\infty}$ should be reanalyzed statistically.

11. The LLOQ for chlorthalidone was 50 ng/ml, but the Low QC was 200 ng/ml or four times the LLOQ. For future studies, the sponsor should follow the Division's recommendation that the Low QC not exceed three times the LLOQ.

(b)5 - Gov't Pre-Decisional

XII. Recommendations

1. The bioequivalence study conducted by Sidmak Laboratories on its atenolol/chlorthalidone 100/25 mg tablet, lot #90-026T, comparing it to ICI's Tenoretic® 100 tablet, lot #DA-161, has been found incomplete by the Division of Bioequivalence. The firm should submit additional data in response to deficiencies 1-10 above.

2. The sponsor should resubmit its waiver request for the test product atenolol/chlorthalidone 50/25 mg tablet with its response to the deficiencies above.

3. The sponsor should be informed of deficiencies 1-11 and recommendations 1-2.

/S/

James D. Henderson, Ph.D.
Review Branch II
Division of Bioequivalence

RD INITIALED FPELSOR /S/
FT INITIALED FPELSOR

Concur: /S/ Date 6/6/92
Shrikant V. Dighe, Ph.D.
Director
Division of Bioequivalence

JDH/sdl/5-22-92/74107

cc: ANDA #74-107, original, HFD-630, HFD-604 (Hare), HFC-130 (JAllen), HFD-655 (Tran, Henderson), Drug File

Table 1. In Vitro Dissolution Testing

Drug (Generic Name): atenolol / chlorthalidone
Dose Strength: 100/25 mg and 50/25 mg
ANDA No.: 74-107
Firm: Sidmak
Submission Date: 8/22/91
File Name: 74107SD.691

I. **Conditions for Dissolution Testing:**

USP XXII Basket: Paddle: X RPM: 50
No. Units Tested: 12
Medium: water Volume: 900 ml
Specifications: atenolol: NLT (b)4 45 min (30 min for FDA)
chlorthalidone: NLT (b)4 45 min
Reference Drug: Tenoretic® 100 and Tenoretic® 50 (ICI Pharma)
Assay Methodology: (b)4 -

II. **Results of In Vitro Dissolution Testing:**

Sampling Times (Minutes)	Test Product Lot #90-026T Strength(mg) 100/25			Reference Product Lot #DA-161 exp 2/94 Strength(mg) 100/25		
Atenolol	**Mean %**	**Range**	**%CV**	**Mean %**	**Range**	**%CV**
15	93.9	(b)4 - Confidential Business	2.9	91.6	(b)4 - Confidential Business	5.3
30	96.3		2.0	97.0		5.0
45	97.8		1.6	99.4		3.4

Sampling Times (Minutes)	Test Product Lot #90-026T Strength(mg) 100/25			Reference Product Lot #DA-161 Strength(mg) 100/25		
Chlorthal.	**Mean %**	**Range**	**%CV**	**Mean %**	**Range**	**%CV**
15	100.6	(b)4 - Confidential Business	4.6	87.0	(b)4 - Confidential Business	5.6
30	101.9		1.9	93.5		6.2
45	102.9		1.8	96.4		5.2

Sampling Times (Minutes)	Test Product Lot #90-024T Strength(mg) 50/25			Reference Product Lot #4131H exp 1/91 Strength(mg) 50/25		
Atenolol	**Mean %**	**Range**	**%CV**	**Mean %**	**Range**	**%CV**
15	93.3	(b)4 - Confidential Business	4.5	101.5	(b)4 - Confidential Business	2.9
30	95.7		4.2	102.8		1.3
45	102.0		4.2	104.0		1.4

Sampling Times (Minutes)	Test Product Lot #90-024T Strength(mg) 50/25			Reference Product Lot #4131H exp 1/91 Strength(mg) 50/25		
Chlorthal.	**Mean %**	**Range**	**%CV**	**Mean %**	**Range**	**%CV**
15	97.8	(b)4 - Confidential Business	4.9	94.6	(b)4 - Confidential Business	5.2
30	99.5		4.7	98.8		2.7
45	105.7		4.9	100.0		2.5

Table 2 - Intraday Precision and Accuracy

Sample	Nominal Conc. (ng/ml)	Det'd. Conc. (ng/ml)	CV (%)	Accuracy (%) Mean	Range	N
Atenolol						
LLOQ	30.0	31.61	8.9	105.4	(b)4 - Confidential Business	10
Low QC	90.0	86.81	4.9	96.5		10
Medium QC	600.0	570.06	3.3	95.0		10
High QC	1000.0	975.32	3.3	97.5		10
Chlorthalidone						
LLOQ	50.0	46.42	7.1	92.8		6
Low QC	200.0	201.87	3.1	100.9		6
Medium QC	3500.0	3688.4	1.1	105.4		6
High QC	8000.0	8218.58	1.8	102.7		6

Table 4 - Mean Blood Levels of Atenolol as Reported by the Sponsor (N = 24)

Time (hr)	Trt. A (test) Mean (ng/ml)	CV (%)	*	Trt. B (ref.) Mean (ng/ml)	CV (%)	*	% Diff.
0	0.0	-	0	0.0	-	0	-
0.5	169.06	34.2	23 **	139.15	50.5	24	21.5
1	409.15	32.2	24	437.38	32.3	24	-6.4
1.5	507.16	39.3	24	565.63	38.7	24	-10.3
2	524.42	38.8	24	588.12	41.2	24	-10.8
2.5	509.83	33.0	24	610.09	37.9	24	-16.4
3	528.37	29.3	24	614.01	36.3	24	-14.0
4	514.89	36.3	24	586.12	40.8	24	-12.2
5	464.15	40.7	24	501.78	38.3	24	-7.5
6	366.24	35.6	24	409.99	40.1	24	-10.7
8	266.93	34.4	24	294.03	37.3	24	-9.2
10	198.75	34.3	24	223.06	33.2	24	-10.9
12	150.28	33.6	24	162.00	30.6	24	-7.2
18	73.58	27.8	24	78.63	27.5	24	-6.4
24	42.00	47.2	19 ***	43.14	50.1	17 ****	-2.8
36	3.93	310.6	2 ****	3.61	317.2	2 *****	8.9

* Number of nonzero concentrations
** one missing value
*** two missing values
**** four missing values
***** three missing values

Table 5 - Mean Pharmacokinetic Parameters of Atenolol as Reported (N = 24)

Parameter		Trt. A (test) Mean	CV(%)	Trt. B (ref.) Mean	CV(%)	% Diff.	90% CI
AUC(0-t)	ng-hr/ml	5099.1	30.5	5601.42	32.9	-8.97	84.7-97.4
AUCINF	ng-hr/ml	5515.4	28.8	6039.41	31.1	-8.68	85.4-97.2
CMAX	ng/ml	630.0	31.7	702.9	33.3	-10.37	81.6-97.7
TMAX	hr	2.98	41.5	2.96	35.9	0.68	-
KEL	1/hr	0.12	17.0	0.126	17.9	-4.76	-
T1/2	hr	5.76	-	5.58	-	-	-

Trt. A = Atenolol/Chlorthalidone 100/25 mg (Sidmak)
Trt. B = Tenoretic 100/25 mg (ICI)

Table 6 - Mean Blood Levels of Chlorthalidone as Reported by the Sponsor (N = 24)

Time (hr)	Trt. A (test) Mean (ng/ml)	CV (%)	*	Trt. B (ref.) Mean (ng/ml)	CV (%)	*	% Diff.
0	0.0	-	1**	0.0	-	1**	-
0.5	116.46	119.6	17	46.28	106	14	151
1	566.23	43.1	24	361.51	37.6	24	56.6
2	1321.07	25.8	24	1038.72	26.3	24	27.2
3	1687.25	20.3	24	1442.07	20.4	24	17
4	1884.75	17.2	24	1707.7	18.4	24	10.4
6	2019.24	17.3	24	1917.34	20	24	5.3
8	2032.14	16.8	24	1941.05	19.8	24	4.7
10	2042.61	16.4	24	1939.58	18.3	24	5.3
12	2046.51	15.6	24	1889.05	18	24	8.3
14	1971.67	14.7	24	1903.27	19.4	24	3.6
16	1905.55	15.2	24	1831.79	18.8	24	4.0
18	1882.35	15.4	24	1759.26	17.9	24	7.0
24	1799.15	16.7	24	1684.73	17.6	24	6.8
36	1461.35	18.4	24	1394.01	19.1	24	4.8
48	1242.94	17.7	24	1227.27	20.5	24	1.3
72	856.06	21.8	24	827.46	22.1	24	3.5
96	602.35	32.7	24	604.35	26.5	24	-0.3
120	431.58	29.1	24	425.38	29.3	23	1.5

* Number of nonzero concentrations
** Set to 0.0 for statistics

Table 7 - Mean Pharmacokinetic Parameters of Chlorthalidone as Reported (N = 24)

Parameter		Trt. A (test) Mean	CV(%)	Trt. B (ref.) Mean	CV(%)	% Diff.	90% CI
AUC(0-t)	ng-hr/ml	134381	18.6	128186	19.5	4.8	101.2-108.5
AUCINF	ng-hr/ml	165576	22.3	160016	22	3.5	99.8-107.1
CMAX	ng/ml	2125.6	16.3	2006	18.8	6.0	102.7-109.2
TMAX	hr	9.58	25.4	10.42	24.7	-8.1	-
KEL	1/hr	0.015	16.1	0.014	16.1	7.1	-
T1/2	hr	47.02	-	48.8	-	-	-

Trt. A = Atenolol/Chlorthalidone 100/25 mg (Sidmak)
Trt. B = Tenoretic 100/25 mg (ICI)

Table 8 - Determination of KE for Subjects 5 and 6

Parameter	Subject 5, Per. 1	Subject 6, Per. 1
Volume (L)[1,2]		
CS[3]	10.24	8.005
NR[3]	9.627	6.202
KA (hr^{-1})		
CS	0.289	0.46
NR	0.367	0.492
KE (hr^{-1})		
CS	0.01969	0.01278
NR	0.01901	0.01290
TL (hr)		
CS	0.074	0.0426
NR	0.299	0.283
Terminal $T_{1/2}$ (hr)		
CS	35.195	54.206
NR	36.471	53.753
Washout Period (# of half-lives)	9.21	6.25

[1] CS = initial estimates from curve-stripping

[2] The PKCALC program does not directly estimate volume (V). Volume (in liters) is estimated from the equation

$$V = F * D / (AUC * KE)$$

where F = oral availability (0.64 from Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th ed., p. 1669)

D = dose of 25 mg

AUC from the trapezoidal rule, converted to units of mg-hr/L

KE = terminal phase rate constant (hr^{-1})

[3] NR = nonlinear regression

Table 9 - Calculation of AUCINF for Atenolol and Chlorthalidone

	Trt. A (test)		Trt. B (ref.)		% Diff.	90% CI
	Mean	CV(%)	Mean	CV(%)		
Atenolol						
$AUC_{0-\infty}$ (ng-hr/ml)	5508.11	29.62	6019.62	32.43	-0.085	85.5-97.5
$logAUC_{0-\infty}$	-	-	-	-	-	99.4-107.8
RATIO (range)	0.9088	4.57	0.9128	4.2	-0.44	-
KEL (hr^{-1})	0.1085	18.88	0.1135	15.41	-3.7	-
$T_{½}$ (hr)	6.729	30.11	6.37	24.98	5.64	-
NUMHALF (range)	3.67	15.78	3.76	15.84	-2.39	-
Chlorthal.						
$AUC_{0-\infty}$ (ng-hr/ml)	166698	22.44	161044	21.9	3.51	99.8-107.2
$logAUC_{0-\infty}$	-	-	-	-	-	99.4-107.8
RATIO (range)	0.8147	5.74	0.803	6.02	1.46	-
KEL (hr^{-1})	0.0144	16.12	0.01396	16.4	3.15	-
$T_{½}$ (hr)	49.42	16.65	51.04	17.61	-3.17	-
NUMHALF (range)	2.49	16.12	2.40	16.91	3.75	-

Trt. A = Atenolol/Chlorthalidone 100/25 mg (Sidmak)
Trt. B = Tenoretic® 100/25 mg (ICI)

MAY 20 1993

Atenolol/Chlorthalidone
100 mg/25 mg, 50 mg/25 mg tablets
ANDA #74-107
Reviewer: James D. Henderson
File: 74107SDW.N92

Sidmak Laboratories
East Hanover, NJ
Submitted:
November 4, 1992 &
January 4, 1993

Response to Review of a Bioequivalence Study

I. Background

On 8/22/91 the sponsor submitted the results of a bioequivalence study comparing its test product atenolol/chlorthalidone 100/25 mg tablet (ANDA #74-107) with the reference product Tenoretic® 100 (ICI). In addition, the sponsor requested waiver from bioequivalence study requirements for its test product atenolol/chlorthalidone 50/25 mg tablet and submitted dissolution data for both strengths. The submission was reviewed (received 4/13/92, file date 6/9/92), found incomplete, and the sponsor informed of the deficiencies (6/30/92). In the present submission, the firm has responded to the deficiency comments. In response to a request for additional information, the firm submitted a second amendment on 1/4/93 (received 2/1/93).

II. Responses to Deficiency Comments

Deficiency 1: The sponsor must provide an executed batch record for the biostudy lot of test product (#90-026T) which indicates both the theoretical and finished batch sizes.

Response: A copy of the executed batch record is attached. For biobatch #90-026T, the theoretical batch size was [redacted](b)4 - [redacted] the finished batch size was [redacted](b)4 - [redacted] its. Manufacturing dates were 11/30/90 through 12/7/90.

Comment: Waiver from minimum batch size requirements will be required. The sponsor subsequently submitted (1/4/93) a formal request for waiver from biostudy batch size requirements (record of conversation attached).

Deficiency 2: The sponsor must provide documentation showing the potencies of both test and reference biostudy products.

Response: For the test product biobatch 90-026T, potencies were 99.8% and 101.2% for atenolol and chlorthalidone, respectively. For the reference product biostudy lot #DA-161, potencies were 99.3% and 100.0% for atenolol and chlorthalidone, respectively.

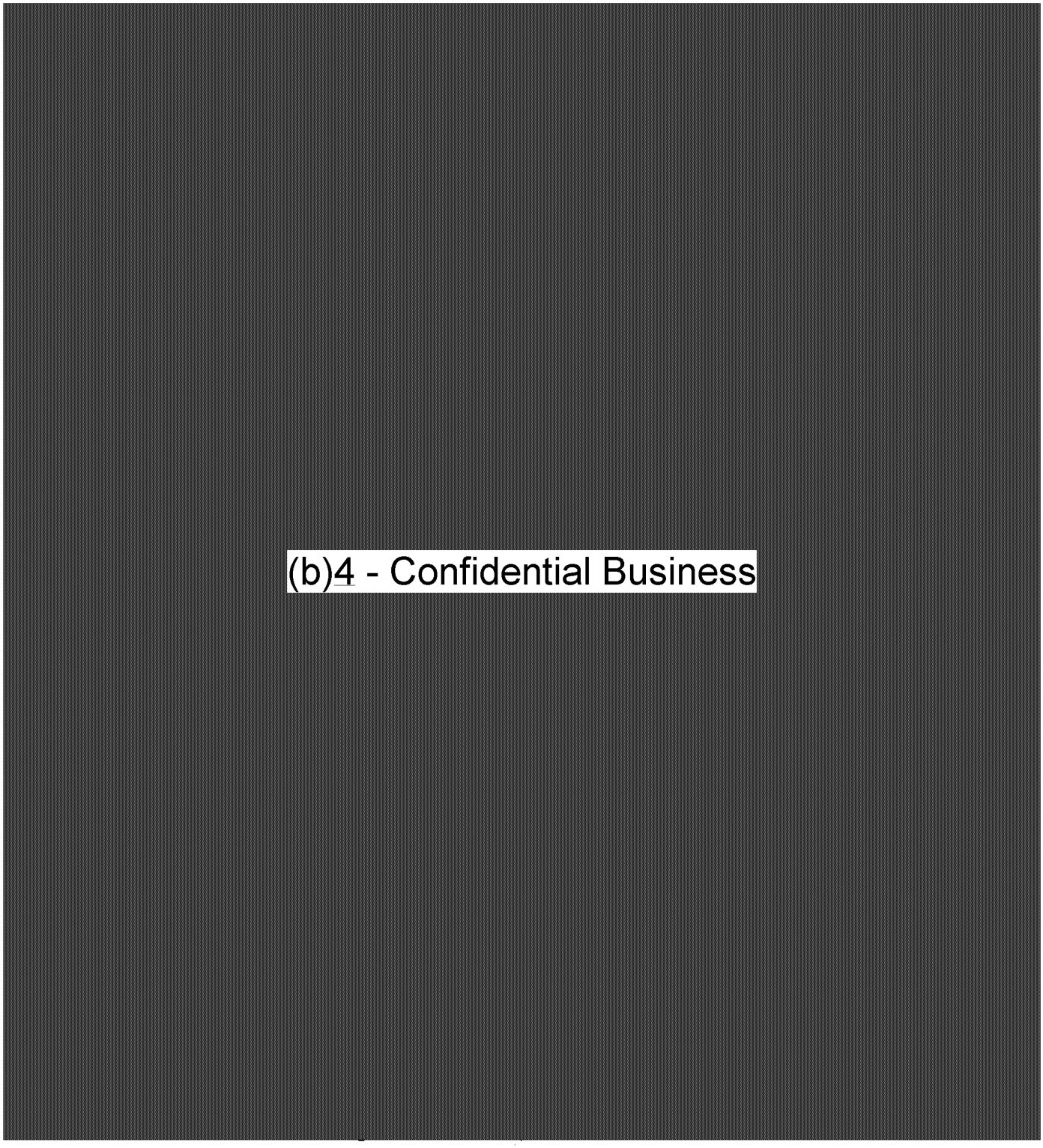
Comment: Acceptable.

Deficiency 3: The sponsor should include raw data for the results of autosampler stability for both analytes.

(b)4 - Confidential Business

Comment: Data for autosampler stability is summarized below; the % deviations are calculated by the reviewer from nominal concentrations.

(b)4 - Confidential Business



Comment: Acceptable.

Deficiency 6: The sponsor should explain its rationale and provide all data and calculations concerning the choice of 1/CONC as weighting factor for standard curves for both analytes.

Response: The simplest algorithm considered at Phoenix is 1/c, consistent with the SOP.

Comment: The sponsor provided no data or calculations to support its choice of weighting factor. From the previous review (file date 6/9/92), the reviewer noted that slightly higher correlations between PHR vs. concentration were obtained using 1/c instead of $1/c^2$ as weighting factor although $1/c^2$ appeared to be more highly correlated with 1/variance. In the absence of Division or OGD policy, the reviewer accepts the sponsor's choice of weighting factor.

Deficiency 7: The sponsor should explain why the 50 ng/ml and 1500 ng/ml standards from chlorthalidone standard curve ANQ03 were not rejected since their back-calculated values deviate from nominal values by -36% and 20.7%, respectively. Similarly, the 50 ng/ml and 100 ng/ml standards from curve ANQ05 deviate by -24.8% and 28.9%, respectively. The sponsor should explain why these standards were also not rejected.

Response: For chlorthalidone standard curve ANQ03, r = 0.9976 and all six standards were acceptable according to the SOP. For ANQ05, r = 0.9959 and 5/6 standards were acceptable. Standards are rejected at Phoenix only if there is evidence, from back-calculated QC's or correlation coefficients, that they are biasing the standard curve unduly.

Comment: Although the sponsor states that back-calculated standard concentrations are not used for data acceptance, tables of these values were submitted for both analytes. The reviewer counts seven standards in the table (T2) of chlorthalidone back-calculated calibration standard concentrations: 50, 100, 500, 1500, 5000, 9000, and 10000 ng/ml. For curve ANQ05, there is no indication in this table that one of the standards was rejected. As stated in the previous review (file date 6/9/92), those standards listed above should have been rejected in the reviewer's opinion.

On further examination of the sponsor's originally reported data and the reviewer's revised calculations for the two standard curves above, the reviewer discovered transcriptional errors in the table on p. 10 of the previous review. The table should be corrected as follows:

Subj.	Trt., Per.	AUC-reported	AUC-revised	CMAX-reported	CMAX-revised
7	1 , 1	139002	140768	2409.1	2420.34
7	2 , 2	140060	141823	2418.7	2429.91

The 90% CI for AUC_{0-t}, C_{max}, and their log-transformed values as calculated by the reviewer are shown in Table 1 (attached) under several conditions ($AUC_{0-\infty}$ will be discussed in the response to deficiency #10 below). Condition 1 shows the results for the sponsor's original data with all subjects as calculated by the reviewer. Condition 3 shows the results after using the revised standard curves ANQ03 and ANQ05 and the recalculated data for Subjects 1,2,5,6,7.

Deficiency 8: The sponsor should explain the presence of nonzero predose chlorthalidone levels in subject 5 for both periods of the study.

Response: The predose concentrations for S5 in both periods are most likely due to an endogenous interfering substance. For Period 2, the predose concentration (61.6 ng/ml) is not likely to be chlorthalidone based on the terminal phase $t_{\frac{1}{2}}$ from Period 1 (33.88 hr) and the 9-day interval (6.38 half-lives) between C_{LAST} (151.3 ng/ml) from Period 1 and 0 hr of Period 2 (extrapolated concentration of 2.01 ng/ml). Statistical analysis of chlorthalidone data was repeated after exclusion of S5. The 90% CI were: AUC_{0-t}, 101.5-108.9; $AUC_{0-\infty}$, 99.9-107.4; and C_{max}, 103.0-109.6.

Comment: If the predose levels for Subject 5 in both periods are due to an interfering substance from the subject's plasma, then it is possible that the interference was present throughout both sampling periods. Condition 2, in Table 1 (attached) shows the 90% CI results from the sponsor's data after Subject 5 is deleted. Conditions 3 and 4 in Table 1 show the 90% CI results after revision of standard curves ANQ03 and ANQ05 by the reviewer with and without Subject 5, respectively.

One consequence of rejecting the standards listed above from curve ANQ05 (deficiency #7) and recalculating the plasma chlorthalidone concentrations was that S6 then has a nonzero predose level (65.13 ng/ml) in Period 2. Condition 5 in Table 1 shows the 90% CI results with both Subjects 5 and 6 deleted using the revised plasma level data.

The concentration range for revised standard curve ANQ05 is 500-10000 ng/ml since the two lowest standards (50 and 100 ng/ml) should have been rejected due to their large deviations from nominal concentrations. It should be noted that the results in

Table 1 from Conditions 3-5 use all of the recalculated plasma level data from standard curve ANQ05 for Subjects 5, 6, and 7, including those plasma levels < 500 ng/ml. Therefore, a final recalculation for AUC was done using only the plasma levels > 500 ng/ml from revised curve ANQ05 for Subjects 5, 6, and 7. These "AUC->500" values are shown in the table below:

Subj, Per, Trt	AUC-revised	AUC->500
5,1,1	79351	50630
5,2,2	84374	52888
6,1,1	156597	156675
6,2,2	160214	160510
7,1,1	140768	129552
7,2,2	141823	129021

The 90% CI are shown in Condition 6 in Table 1.

Deficiency 9: The sponsor should explain the observed statistically significant ($p < 0.1$) sequence effects for chlorthalidone AUC and CMAX.

Response: A statistically significant sequence effect occurs about 10% of the time even when there are no true sequence effects or residual effects when testing is done at the 10% level. Based on the Division of Bioequivalence Statistical Guidance, a sequence effect is acceptable if the study is single dose, uses only normal volunteers, the drug is not endogenous, and there are no predose levels in Period 2. These criteria are met except for the presence of predose levels in Subject 5 in both periods. As stated above, these predose levels are most likely due to some interfering substance and a more than adequate washout period was used for this subject.

Comment: In reference to the results shown in Table 1, there were statistically significant ($p < 0.1$) sequence effects for AUC_{0-t}, C_{max}, and their log-transformed values for Conditions 1, 3, 5, and 6. Deleting Subject 5 from the sponsor's original data (Condition 1 → Condition 2) removes the sequence effect although the p values are still somewhat low (0.1183-0.145). Similarly, deleting Subject 5 from the revised data (Condition 3 → Condition 4) also removes the sequence effect ($p = 0.1279$-0.1456). Inspection of the original data shows that Subject 5 (Sequence 1) has comparatively low responses for AUC and C_{max} for both treatments which could be contributing to the sequence effect.

The key question is whether or not the predose level for Subject 5 in Period 2 represents carryover from Period 1. In the

previous review (file date 6/9/92), the reviewer used nonlinear regression to estimate the terminal elimination rate constant for Subject 5 from Period 1 data. The terminal phase $t_{\frac{1}{2}}$ was calculated as 36.5 hr; therefore, the washout interval (two weeks between dosings) was 9.2 half-lives. An adequate washout exists and the predose level for Subject 5 is most likely due to interference from some substance in plasma, particularly in view of the predose level in Period 1.

The situation for Subject 6 is less clear. Using the revised standard curve data from curve ANQ05, Subject 6 has a nonzero predose level in Period 2. The terminal phase $t_{\frac{1}{2}}$ from Period 1 for Subject 6 was 53.75 hr by nonlinear regression, or 6.25 half-lives. This may or may not be an adequate washout. Using the sponsor's original data and standard curve parameters, the peak height response for sample 6-0-2 was 43, which calculates as 40.35 ng/ml. Although this value is BLQ (50 ng/ml), it is still a significant response and could possibly represent some carryover. Inspection of the sponsor's analytical raw data shows eleven instances where integrated peaks were detected in Period 2 predose samples (Subjects 1, 2, 5, 6, 10, 14, 16, 17, 21, 23, 25) with peak height responses from 19-52. Whether or not these predose peaks could represent carryover from Period 1 is discussed in the comment to deficiency #10.

> **Deficiency 10: The sponsor's reported chlorthalidone data indicates mean values of approximately 0.80 for both treatments for the ratio $AUC_{0-TLAST}$ / $AUC_{0-\infty}$, and that blood sampling occurred over approximately 2.5 half-lives (120 hr / 50 hr) for both treatments. Therefore, the sponsor should use state-of-the-art nonlinear regression methods for accurate determination of the KEL values used to calculate $AUC_{0-\infty}$ for each data set. The revised values of $AUC_{0-\infty}$ should reanalyzed statistically.**

Response: The FDA statistical guidance indicates that the KEL value should be calculated with an appropriate method which, traditionally, has been linear least squares regression using the last three or more points. This is consistent with the model-independent approach towards bioequivalence calculations.

Comment: The reviewer performed nonlinear least squares regression analysis of the chlorthalidone data sets as follows:

- The ESTRIP function of the PKCALC program (Shumaker, Drug Metab Rev 1986;17:331) was used to strip each data set into a sum of two exponentials and generate initial estimates for pharmacokinetic parameters. The reviewer used the revised data sets for Curve ANQ03 (Subjects 1,2) and Curve ANQ05 (Subjects 5,6,7), including those plasma levels < 500 ng/ml for Subjects 5, 6, and 7.

- Literature reports (Beermann, Clin Pharmacokinet 1980;5:221) indicate that chlorthalidone is 50-80 times more highly concentrated in RBC's than in plasma due to high affinity for erythrocyte carbonic anhydrase. Consequently, the plasma kinetics of chlorthalidone after a single oral dose are described by a two-compartment model (three exponential terms) with terminal phase half-lives of 40-65 hr. However, the whole blood kinetics (uptake, nonlinear binding, and elimination in RBC's) of chlorthalidone are described by a one-compartment model (two terms) with terminal phase half-lives of 53-60 hr.

- The uptake of chlorthalidone in RBC's is saturable and saturation has been reported to occur at whole blood concentrations *in vitro* of 15-20 µg/ml (Dieterle, Eur J Clin Pharmacol;1976;10:37). The 25 mg dose of chlorthalidone in this study produced *in vivo* maximum C_{max} values of 2830 ng/ml (2.83 µg/ml) and 2670 ng/ml (2.67 µg/ml) for test and reference products, respectively, which are far below the whole blood concentrations required for nonlinear binding.

- PCNONLIN v. 3.0 was then used to fit (simplex method) each data set to a one-compartment model with first-order input and time lag using the initial estimates for the absorption rate constant (KA), the elimination rate constant (KE), time lag (TL), and a calculated estimate for volume of distribution (V). Final estimates were then produced for KE, $t_{\frac{1}{2}}$, and $AUC_{0-\infty}$ and are shown in Table 2 (attached). The number of half-lives over which sampling occurred (NUMHALF = t_{LAST} / $t_{\frac{1}{2}}$), the ratio of trapezoidal AUC to extrapolated AUC (RATIO = AUC_{0-t} / $AUC_{0-\infty}$), and the number of half-lives during the washout period between treatments (WASHOUT = 336 / $t_{\frac{1}{2}}$) were calculated.

- In the Comment to Deficiency #9 above, the reviewer noted eleven instances of detectable peaks in the Period 2 predose samples. The WASHOUT for these eleven cases ranged from 5.3-9.2 half-lives with 9/11 cases having WASHOUT $\leq 7 * t_{\frac{1}{2}}$. The reviewer calculated the extrapolated predose concentration for Period 2 (C_{PRE2}) using the sponsor's reported last concentration from Period 1 (C_{LAST1}), the KE values reported by the sponsor and determined by the reviewer from the PCNONLIN fitting procedure, and the time interval of 216 hr (washout interval of 336 hr minus t_{LAST} of 120 hr from Period 1) as follows:

$$C_{PRE2} = C_{LAST1} * EXP(-KE * 216)$$

The results are shown in Table 3 (attached). In the case of Subject 23, the C_{PRE2} actually exceeded the LOQ (50 ng/ml).

However, all the remaining samples quantitated to BLQ.

- The fitted compartmental values of $AUC_{0-\infty}$ and $logAUC_{0-\infty}$ were then analyzed by the GLM procedure of SAS and the 90% CI calculated: $AUC_{0-\infty}$, 99.7-107.8; $logAUC_{0-\infty}$, 99.3-108.3. There were no statistically significant sequence, period, or treatment effects.

- Since blood levels < 500 ng/ml are in question for revised curve ANQ05, the reviewer repeated the calculation above excluding Subjects 5, 6, and 7. The 90% CI were 99.8-109.1 for $AUC_{0-\infty}$ and 99.5-109.9 for $logAUC_{0-\infty}$.

Deficiency 11: The LLOQ for chlorthalidone was 50 ng/ml, but the Low QC was 200 ng/ml or four times the LLOQ. For future studies, the sponsor should follow the Division's recommendation that the Low QC not exceed three times the LLOQ.

Response: Subsequent to this study, the SOP has been changed to reflect this recommendation.

Comment: Acceptable

III. Waiver Request

1. In the previous review (file date 6/9/92), the dissolution testing for the lower strength of the test product atenolol/chlorthalidone 50/25 mg tablet was found acceptable using the FDA method and specifications.

2. The formulations for the two test products are exactly proportional with regard to the active ingredient atenolol and the inactive ingredients and contain the same amount of chlorthalidone.

3. The lower strength of the test product atenolol/chlorthalidone 50/25 mg tablet is therefore eligible for waiver of *in vivo* bioequivalence study requirements as stated in 21 CFR Section 320.22(d)(2)(i-iii) of the Bioavailability/Bioequivalence Regulations.

IV. Conclusions

1. From Table 1 it is apparent that the 90% CI for chlorthalidone AUC_{0-t}, C_{max}, and their log-transformed values are all within the allowed equivalence intervals of 80-120% (untransformed) or 80-125% (log-transformed) of the reference mean. This is the case using the sponsor's original data (Condition 1), excluding Subject 5 due to interferences in the predose samples from both periods

(Condition 2), or using the revised data calculated by the reviewer (Condition 3) and other variations of this data (Conditions 4-6).

2. The predose levels in Subject 5 samples from both periods are most likely due to an interfering substance in that subject's plasma since: 1) the calculated plasma levels from the revised standard curve are about the same in both periods (Period 1, 90 ng/ml; Period 2, 79 ng/ml); 2) a washout of 9.2 half-lives occurred between treatments; 3) the predicted concentration for the Period 2 predose sample is about 2-2.5 ng/ml (Table 3).

3. From the sponsor's analytical raw data, there were eleven instances of detectable peaks in the Period 2 predose samples. In 9/11 cases, the WASHOUT was $\leq 7*t_{\frac{1}{2}}$ (range 5.3-7.0 half-lives). The predicted concentrations at the start of Period 2 (Table 3) may possibly represent some amount of carryover. However, the Division does not rely upon extrapolated or predicted concentrations for conclusions regarding bioequivalence. All of these predose samples quantitated to BLQ using the sponsor's original data except for Subject 5.

4. The statistically significant ($p < 0.1$) sequence effects for chlorthalidone $AUC_{0-\infty}$, C_{max}, and their log-transformed values are removed if Subject 5 is deleted from the analysis.

5. The 90% CI for $AUC_{0-\infty}$ and $\log AUC_{0-\infty}$ are within the allowed equivalence intervals using either the KE values determined by the sponsor and the noncompartmental calculation or the $AUC_{0-\infty}$ values determined by the reviewer from the nonlinear regression fitting to a one-compartment model.

6. In the previous review (file date 6/9/92) an analytical protocol deviation was noted for the Period 2, 96-hr samples. The reviewer calculated the % difference between the chlorthalidone blood concentration at 96 hr produced by the Period 2 treatment and the Period 1 treatment (used as reference for this calculation). In 13/25 cases, the Period 2 treatment was higher at 96 hr; in 5/13 of these cases, the difference was > 20%. The analytical protocol deviation does not appear to have significantly affected the results.

7. The sponsor has successfully answered the deficiencies.

8. In the guidance "Statistical Procedures for Bioequivalence Studies Using a Standard Two-Treatment Crossover Design" issued 7/1/92 by the OGD, there were five conditions for acceptance of two-way crossover studies having statistically significant sequence effects. One of these criteria was

that "... in the second phase, the predose biological matrix samples do not exhibit any detectable drug level in all subjects...".

Using the sponsor's originally reported data including all subjects, there were statistically significant ($p < 0.1$) sequence effects for AUC_{0-t}, C_{max}, and their log-transformed parameters. In addition, Subject 5 had a nonzero predose level in Period 2. These observations constitute a violation to the five conditions required by OGD for approval as stated in the Statistical Guidance (7/1/92). Therefore, OGD would have to concur with the following rationale for acceptance:

- An adequate washout (9.2 half-lives) for Subject 5 occurred.
- The nonzero predose level for Subject 5 from Period 1 supports the argument that an interfering substance is causing the predose levels from both periods.
- The presence of an interfering substance in Subject 5 samples from both periods favors deletion of this subject from the final results.

9. Before final approval of the application, the sponsor must submit a formal request for waiver from bioequivalence study batch size requirements in view of its finished batch size of (b)4 units for the test product atenolol/chlorthalidone 100/25 mg tablet, lot #90-026T. The waiver request was submitted on 1/4/93 (record of conversation attached).

V. Corrections from Review of File Date 6/9/92

While checking this review in preparation for final form, errors were discovered in Table 9 in the previous Division review of file date 6/9/92. The 90% CI values for revised $AUC_{0-\infty}$, $\log AUC_{0-\infty}$, and RATIO for atenolol were generated from a data set entered by the reviewer containing four incorrect values for revised AUC_{0-t}. These entries were changed to correspond with the correct revised AUC values for Subjects 16 and 17 shown in the table on p. 9 of the 6/9/92 review. The correct 90% CI for $AUC_{0-\infty}$ and $\log AUC_{0-\infty}$ and RATIO values are shown in Table 6 (attached). The conclusions are unchanged.

VI. Recommendations

1. The bioequivalence study conducted by Sidmak Laboratories on its atenolol/chlorthalidone 100/25 mg tablet, lot #90-026T, comparing it to Tenoretic® 100 100/25 mg tablet, lot #DA-161, has been found acceptable by the Division of Bioequivalence. The

study demonstrates that Sidmak's atenolol/chlorthalidone 100/25 mg tablet is bioequivalent to the reference product Tenoretic® 100 100/25 mg tablet manufactured by ICI.

2. The dissolution testing conducted by Sidmak Laboratories on its atenolol/chlorthalidone 100/25 mg tablet, lot #90-026T, is acceptable and should be incorporated into the firm's manufacturing controls and stability program. The dissolution testing should be conducted in 900 ml of distilled water at 37° using USP XXII method with apparatus 2 (paddle) at 50 rpm. The test product should meet the following specifications:

Atenolol: Not less than (b)4 of the labeled amount of drug in the dosage form is dissolved in 30 minutes.

Chlorthalidone: Not less than (b)4 of the labeled amount of drug in the dosage form is dissolved in 45 minutes.

3. From the bioequivalence point of view, the firm has met the requirements of *in vivo* bioequivalence and *in vitro* dissolution testing and the application is acceptable.

4. The dissolution testing conducted by Sidmak Laboratories on its atenolol/chlorthalidone 50/25 mg tablet, lot #90-024T, is acceptable. The firm has conducted an acceptable *in vivo* bioequivalence study (submissions dated 8/22/91, 11/4/92, and 1/4/93) comparing its 100/25 mg tablet of the test product with the 100/25 mg tablet of the reference product Tenoretic® 100 manufactured by ICI. The formulation for the 50/25 mg strength is proportionally similar to the 100/25 mg strength of the test product which underwent bioequivalency testing. The waiver of *in vivo* bioequivalence study requirements for the 50/25 mg strength of the test product is granted. The 50/25 mg strength of the test product is therefore deemed bioequivalent to the 50/25 mg tablet of Tenoretic® 50 manufactured by ICI.

(b)4 - Confidential

James D. Henderson, Ph.D.
Review Branch II
Division of Bioequivalence

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FT INITIALED RPATNAIK

Concur: (b)4 - Confidential Date

Agnes T. Wu, Ph.D.
Acting Director
Division of Bioequivalence